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Status of Waterhyacinth/Hydrilla Infestations and Associated Biological Control Agents in Lower Rio Grande Valley Cooperating Irrigation Districts

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Aquatic Plant Control Research Program

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Final report

Prepared for U.S. Army Corps of Engineers, Washington, DC 20314-1000
U.S. Department of Interior, Bureau of Reclamation, Denver, CO 80225

Engineer Research and Development Center Cataloging-in-Publication Data

Status of waterhyacinth/hydrilla infestations and associated biological control agents in lower Rio Grande Valley cooperating irrigation districts / by Michael J. Grodowitz ... [et al.] ; prepared for U.S. Army Corps of Engineers, U.S. Department of the Interior, Bureau of Reclamation.
41 p. : ill. ; 28 cm. -- (ERDC/EL ; SR-00-11)
Includes bibliographic references.
1. Aquatic weeds -- Biological control -- Rio Grande. 2. Hydrilla -- Biological control -- Rio Grande. 3. Rio Grande. 4. Water hyacinth -- Biological control -- Rio Grande. 5. Lower Rio Grande Valley (Tex.) I. Grodowitz, Michael J. II. United States. Army. Corps of Engineers. III. Engineer Research and Development Center (U.S.) IV. Environmental Laboratory (U.S.) V. United States. Dept. of the Interior. VI. United States. Bureau of Reclamation. VII. Aquatic Plant Control Research Program (U.S.) VIII. Series: ERDC/EL SR ; 00-11.
TA7 E8 no.ERDC/EL SR-00-11

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Preface

The work reported herein was sponsored by the U.S. Department of Interior, Bureau of Reclamation, and by Headquarters, U.S. Army Corps of Engineers (HQUSACE), as part of the Aquatic Plant Control Research Program (APCRP), Work Unit No. 33028. The APCRP is assigned to the U.S. Army Engineer Research and Development Center (ERDC) under the purview of the Environmental Laboratory (EL). Funding for the APCRP was provided under the Department of the Army Appropriation Number 96X3122, Construction General. The APCRP is managed under the Center for Aquatic Plant Research and Technology (CAPRT), Dr. John W. Barko, Director. Mr. Robert C. Gunkel, Jr., was Assistant Director for CAPRT. Program Monitor during this study was Mr. Timothy Toplisek, HQUSACE.

Principal Investigator for this work unit was Dr. Michael J. Grodowitz, Aquatic Ecology Branch, Ecosystem Research Division (ERD), EL, ERDC. This report was reviewed by Drs. Judy Shearer and Alfred F. Cofrancesco. Dr. Michael J. Grodowitz, Ms. Jan E. Freedman, Mr. Harvey Jones, Mr. Lavon Jeffers, and Drs. Carlos F. Lopez and Fred Nibling prepared this report.

While in the Lower Rio Grande Valley, assistance was offered by personnel from the Department of Texas Parks and Wildlife (TPWD), Texas Natural Resource Conservation Commission (TNRCC), International Boundary and Water Commission (IBWC), and participating irrigation districts. The authors would like to especially thank Messrs. Ray Cardona, TPWD, Julian Perales, TNRCC, and Richard Garcia, IBWC, Hidalgo County Irrigation District No. 1, for technical and logistical input.

This investigation was performed under the general supervision of Dr. Conrad J. Kirby, Chief, ERD, and Dr. John W. Keeley, Acting Director, EL, ERDC.

At the time of publication of this report, Dr. James R. Houston was Director of ERDC, and COL James S. Weller, EN, was Commander.

This report should be cited as follows:

> Grodowitz, M. J., Freedman, J. E., Jones, H. L., Jeffers, L., Lopez, C. F., and Nibling, F. (2000). "Status of waterhyacinth/hydrilla infestations and associated biological control agents in Lower Rio Grande Valley Cooperating Irrigation Districts," ERDC/EL SR-00-11, U.S. Army Engineer Research and Development Center, Environmental Laboratory, Vicksburg, MS.

1 Introduction

Over the last 4 to 5 years increasing levels of two noxious aquatic plants, *Hydrilla verticillata* (hydrilla) and *Eichhornia crassipes* (waterhyacinth), have seriously impacted the Lower Rio Grande Valley (LRGV). In 1998, weed infestation were cited as the worst on record for both the Rio Grande and for most, if not all, of the 28 irrigation districts in the LRGV (Chilton 1998). Direct impacts to the LRGV by the presence of these plant species included restricted water delivery, inaccurate water accounting, and an overall breakdown of system maintenance. Infestations of these two weed species, coupled with a prolonged drought in the area, also contributed to observed water losses. For example, the Texas Water Master and the LRGV District Managers Association reported that infestations of waterhyacinth and hydrilla were main contributors to the excessive water loss. Water losses within the LRGV occurred through increased plant evapotranspiration, the use of surging to break through weed dams and then lost as tailwater, and the use of bank storage as a result of water back-ups where weeds blocked canals.

Methods for the control of waterhyacinth and hydrilla primarily include the traditional strategies of chemical and mechanical technologies. While these methods, particularly chemical control procedures, offer excellent short-term control, they offer little in the way of long-term management and necessitate the continual use of these methods in a high-cost, labor-intensive and often environmentally incompatible manner. The development of a true integrated approach for the management of these weed species should incorporate long-term management options as well.

Long-term management options for controlling waterhyacinth and hydrilla mainly includes the use of host-specific insect agents that feed, damage, and subsequently reduce infestations. Three insect agents are available for the management of waterhyacinth. These agents include two weevil species, *Neochetina eichhorniae* (Warner) and *N. bruchi* (Hustache), the mottled and chevroned waterhyacinth weevils, respectively, and *Sameodes albiguttalis* (Warren), the waterhyacinth moth (Perkins 1973, Center and Durden 1981). These species have proven to be highly effective in slowing the growth and stature of waterhyacinth, reducing flowering and hence seed set, and in many circumstances aiding in the total removal of the infestation (Center, Cofrancesco, and Balciunas 1990; Center et al. 1999).

While four insect agents have been released for the control of hydrilla, only one, *Hydrellia pakistanae* (L.f.) Royle, the Asian hydrilla leaf-mining fly, has proven to be effective in reducing the growth and competitive ability of hydrilla (Center et al. 1997, Grodowitz et al. 1997).

The first step in the incorporation of biological control procedures is to initiate surveys for quantifying the existing plant infestation levels, population sizes, and associated damage of the introduced biological control agents. To accomplish this goal, surveys of both waterhyacinth and hydrilla were initiated during September 1999.

The following is a report summarizing the plant/insect surveys initiated during September along selected sections of the Rio Grande within the La Feria Irrigation District, Harlingen Irrigation District, Cameron County No. 1, Hidalgo County Irrigation District No. 1, Cameron County Irrigation District No. 6, and Cameron County Irrigation District No. 2. In addition, information is included on the biology and operational status of the insect agents for waterhyacinth and hydrilla to aid personnel who are not familiar with the agents and their use. Biology/operational status information was taken from the computer-based Noxious and Nuisance Plant Management Information System (PMIS 1998). Copies of this CD as well as one that deals exclusively with aquatic and wetland plants can be obtained free of charge by accessing http://www.wes.army.mil/el/aqua/cdroms.html. We have also included a short section dealing with the steps necessary to incorporate the use of insect biological control into an overall plant management strategy for waterhyacinth and hydrilla on the Rio Grande.

Insect Agents/Waterhyacinth Agents

Overseas surveys were conducted in South America in the 1960s to identify organisms that feed on waterhyacinth in its native range. Three agents were identified: two weevil species in the genus *Neochetina* and one moth species (*Sameodes albiguttalis*). The first agent released was *N. eichhorniae* in southern Florida in 1972 followed by releases of *N. bruchi* and *S. albiguttalis*.

Neochetina eichhorniae **– Mottled Waterhyacinth Weevils and *N. bruchi* - Chevroned Waterhyacinth Weevil**

Adult mottled waterhyacinth weevils (*N. eichhorniae*) are similar in appearance to the chevroned waterhyacinth weevil (*N. bruchi*; Figure 1). Both are usually gray to dark brownish red, with a mottled appearance. In many individuals of the chevroned waterhyacinth weevil, there is a distinct lighter brown to tan chevron (crescent-shaped marking) on the wing covers. Although a distinct chevron can be present in many individuals, it is absent in others; therefore, the dark raised lines present on the wing covers or elytra are mainly used to separate the species. In the mottled

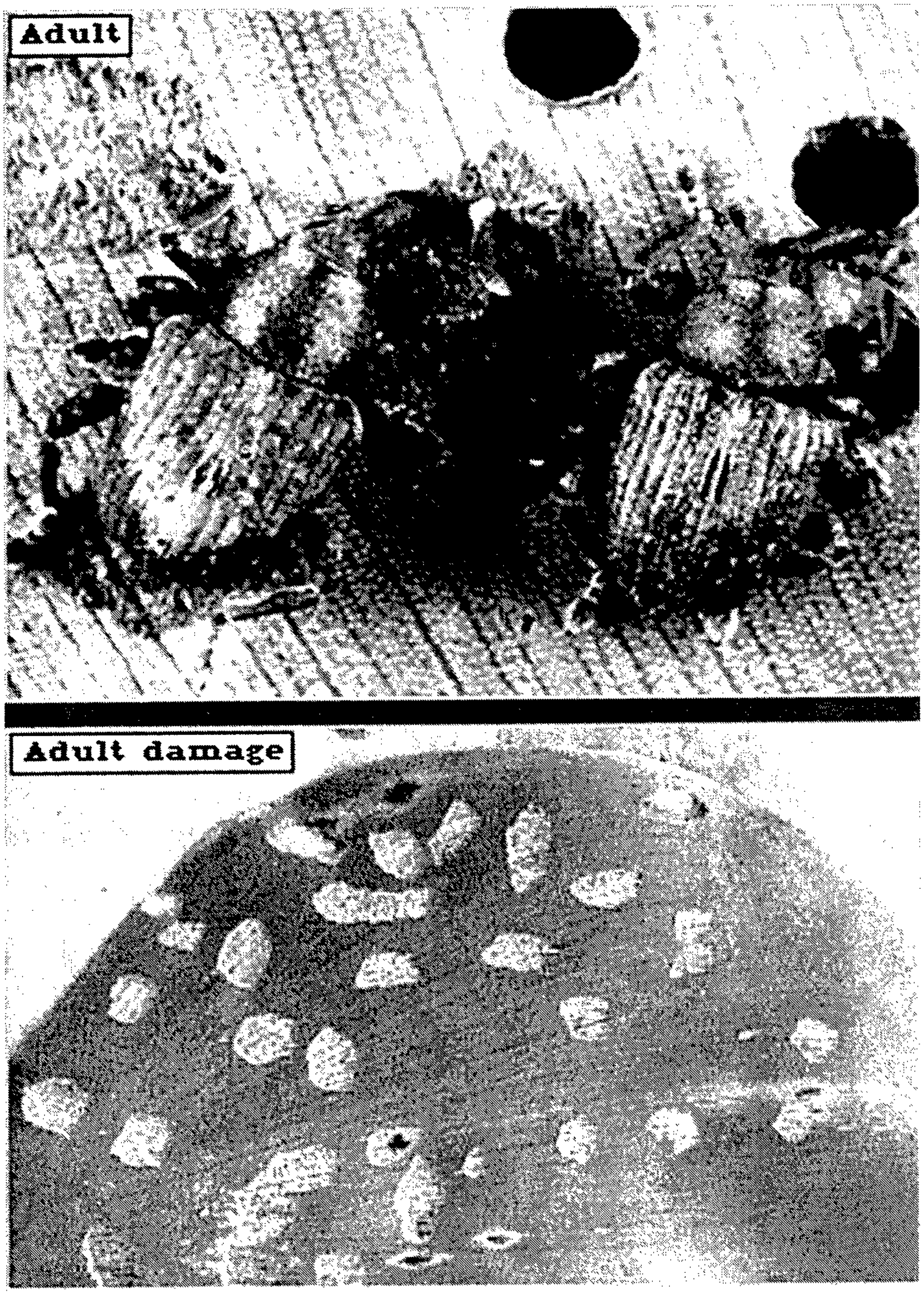

Figure 1. The top photograph contains images of the adults of both *N. eichhorniae* (right) and *N. bruchi* (left). Please note that in *N. bruchi*, the two raised, darkened lines on the elytra are smaller and behind the midline of the wing covers and the vertical striae are less coarse and are more shallow than in *N. bruchi*. The bottom photograph contains an example of the feeding damage produced by both *N. eichhorniae* and *N. bruchi* adults

waterhyacinth weevil, these lines are located forward of the midline of the wing covers, while in *N. bruchi*, the elytra lines are smaller and behind the midline of the wing covers (Figure 1). Another subtle character is the nature of the shallow grooves or striae running the length of the elytra; for the mottled waterhyacinth weevil the striae are relatively "coarse," as opposed to the "fine" striae present on the chevroned waterhyacinth weevil.

Eggs of both weevil species are deposited directly within the tissue of the waterhyacinth plant. Female weevils chew a hole in the lamina or petiole of the leaf and deposit a single egg. Eggs may also be oviposited around the edges of adult feeding scars. It has been reported that mottled waterhyacinth weevils prefer to lay eggs in the tender central leaves or ligules surrounding the leaf bases. Eggs hatch within 7 to 10 days at 75 °F and a single female may oviposit >400 eggs during her lifetime. Most of these eggs (90 percent) are deposited within a single 1-month period.

Larvae are essentially "worm-like," bearing no legs or prolegs and only small enlargements with setae (small hairs) where legs would normally be found (Figure 2). The larvae are usually white or cream-colored with a yellow-orange head. The posterior end of the body is relatively nonspecialized and blunt with a pair of dorsally projecting spiracles that the insect is

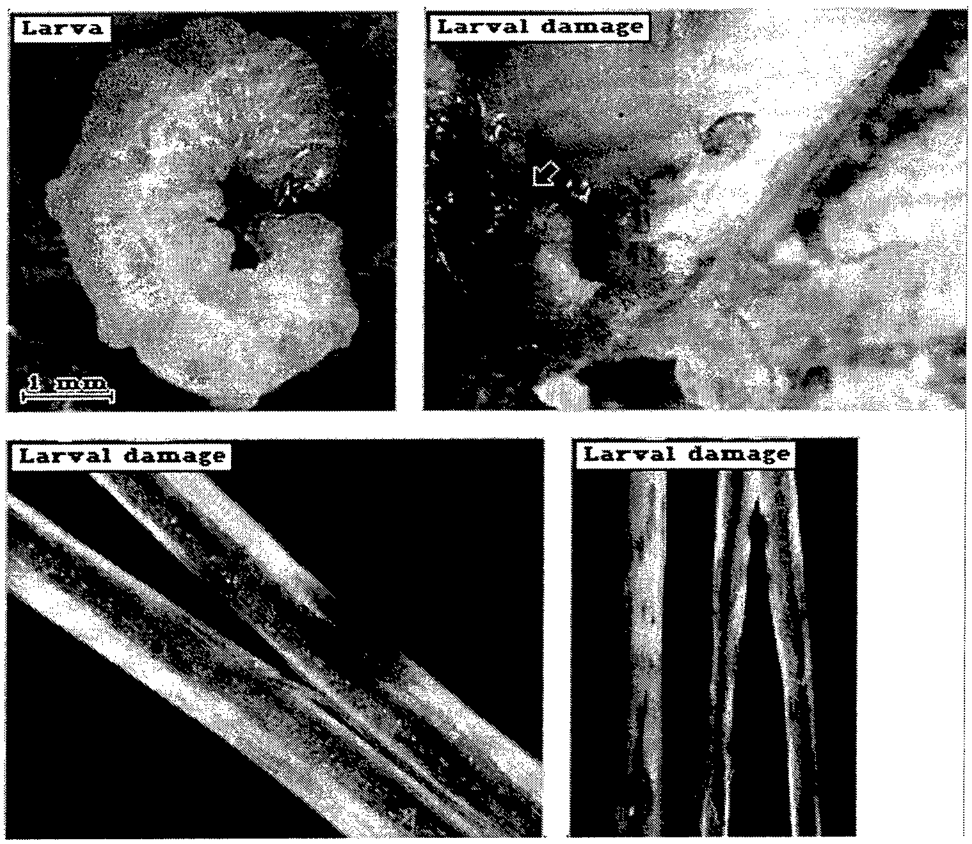

Figure 2. The "grub-like" larvae of both species of *Neochetina* cannot readily be distinguished except by taxonomic experts. Damage by larval *Neochetina* can be found within the plant crown (top right) and within the leaf petioles (bottom row)

thought to insert into the plant tissues to extract oxygen. First-instar larvae are very small (2 mm in length); mature third-instar larvae are "grub-like," C-shaped, and 8 to 9 mm in length. They are virtually indistinguishable (except by experts) from one another. The larvae are typically found feeding within the bases of leaves and petioles, occasionally entering the apex of the stem, where they destroy the apical bud.

Pupae of the waterhyacinth weevils are creamy white and are enclosed within a cocoon that is formed among the lateral rootlets below the water surface (Figure 3). Pupae have the appearance of small "balls" about 5 mm in diameter and are typically found on the roots near the stem. Like the larvae, pupae of the chevroned waterhyacinth weevil are virtually indistinguishable (except by experts) from those of the mottled waterhyacinth weevil.

Figure 3. Pupal case of *Neochetina*. The case is made by the last larval instar where finer root hairs are formed into a small ball using silken threads and then covered on the interior with a layer of silk

Adults of both species are mainly collected by hand or sweep nets and are usually found within the unfurling wrapper leaves and leaf sheaths in great numbers. When population numbers are high, infested plants can be readily moved to new locations. The larvae and pupae are sensitive to handling, and it is not advised to collect these in large numbers for removal to other sites.

Both adults and larvae feed exclusively on waterhyacinth plant tissues. Their damage is virtually indistinguishable from one another. Adult

weevils may be found feeding on the petiole or lamina of the leaf but are more commonly found within the wrapper leaf (i.e., youngest or center leaf) and/or ligule. Adult weevils cause very distinctive damage to waterhyacinth plants. The adults move across the surface of the plant, scraping off small pieces of the leaf epidermis, making short feeding runs, then repeating the movements parallel to the first run. This feeding action continues until a small circular-to-rectangular feeding scar is left. Complete girdling of the petioles is common when large numbers of weevils are present. If adult infestations are high, such damage may significantly impact photosynthetic processes in the leaf. Larval damage is generally restricted to leaf and petiole boring, which can interrupt the movement of nutrients and water within the plant tissues. If larval infestations are heavy, it is not uncommon to see destruction of the apical buds.

While adult and larval feeding may drastically affect the appearance of the plant, the destruction of individual plants and/or overall impact of a population of waterhyacinth plants is not as straightforward. A combination of high populations of both adult and larval weevils can, over a period of time, lead to stunted growth (plants become shorter), decreased flowering, hardening of the plant cuticle, and leaf curling (due to girdling of the leaf and interruption of the flow of plant nutrients and water). Another noticeable feature is the encroachment of other plant species into the waterhyacinth mat (Figure 4). This occurs because meristematic tissues (i.e., new leaves and daughter plants) are destroyed, resulting in less

Figure 4. Waterhyacinth highly stressed by the feeding action of *Neochetina* spp. Note the large number of feeding scars, smaller stature of the plants, curled leaves, lack of flowering, and the presence of other plant species encroaching into what is typically a monotypic infestation of waterhyacinth

overall productivity and growth, permitting slower growing native plants to begin out-competing waterhyacinth in the general area.

Neochetina eichhorniae and *N. bruchi* have proven to be quite effective in reducing the flowering and potential growth of waterhyacinth in the United States. These species are the most widely distributed of the three agents released for the management of waterhyacinth and can be found wherever waterhyacinth is growing. While the damage caused by *N. eichhorniae* and the closely related *N. bruchi* is easily observed, long-term effects to the plant are quite subtle. Rapid and complete control should not be expected as it takes from 3 to 5 years before the effects of these species' feeding can be observed with any regularity. *Neochetina* spp. impact on waterhyacinth is quite indirect. The growth of the plant is reduced to the extent that other, less weedy species, like frogbit, pennywort, etc. can effectively out-compete waterhyacinth, or environmental conditions, such as freezing temperatures, and can knock the plants back to more realistic levels. Such impacts are rarely seen in the absence of *Neochetina* spp. It should be advised that frequent use of chemical applications for waterhyacinth management can adversely affect the ability of the two weevil species to impact the plant. A conscience effort in leaving unsprayed refugia or harborage will allow the buildup of damaging population levels of these two agents.

Sameodes albiguttalis - Waterhyacinth Moth

Sameodes albiguttalis, the waterhyacinth moth, is a pyramid moth native to the Amazon Basin of South America. The moth, which feeds exclusively on waterhyacinth, was released in Florida as a biocontrol agent in 1977.

Adult moths of both sexes are extremely variable in coloration (Figure 5). The forewings of the species range from brown to golden, with the hindwings generally appearing more consistently golden. A distinct white spot is generally present near the leading edge of the forewing, at its midlength. In the center of the hindwing is a distinct dark spot. The distal portions of the abdominal segments are usually white, contributing to the appearance of white rings girdling the abdomen. Sexual dimorphism is moderate, with female moths generally much darker, and slightly larger, than males. While adult moths do not feed on waterhyacinth, they are commonly found resting on the underside of waterhyacinth leaves. The eggs of *S. albiguttalis* are small (ca. 0.3 mm), creamy white, and roughly spherical. The eggs may be irregularly shaped due to their being pushed into various cracks and crevices within the plant by the adult female. Eggs ready for hatching turn a dark brown color because of the maturing larva within the egg. Depending on temperature, eggs take from 3 to 4 days to hatch. Larvae are brown with darker spots at emergence but during larval development, are characterized by a cream-colored body with conspicuous dark brown spots. The brown spots are actually hardened or sclerotized plates that are scattered over the dorso-lateral portions of the body. From these

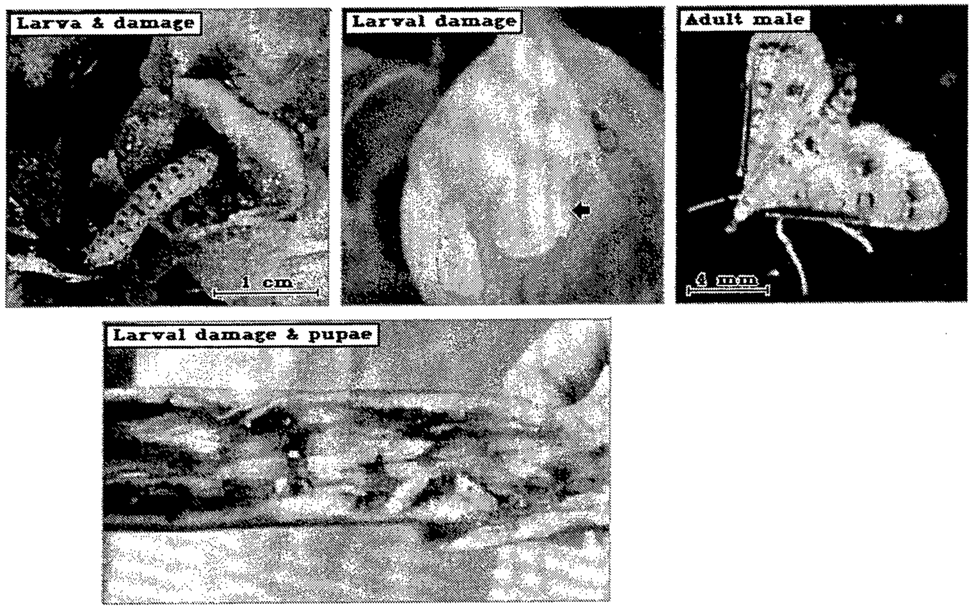

Figure 5. Different life stages of *Sameodes albiguttalis* and associated larval damage

plates arise short, stiff hair-like bristles. Openings to the respiration system (the spiricles) are bordered by a dark brown coloration. First-instar larvae have dark brown to black heads, while later-instar larvae have dark orange heads. Mature larvae are about 18.0 mm long. The pupae of the waterhyacinth moth are characterized by banding of the abdominal segments. The proximal half of these segments is dark brown, while the distal portion is orange. The pupal stage is a quiescent, nonfeeding stage. The pupae remain within the cocoon for 7 to 10 days while undergoing the complex internal changes that lead to the adult form. After metamorphosis is complete, the newly formed adult crawls out the cocoon and exits the petioles. Adult moths generally mate shortly after emerging from the pupal stage and live only a short time (probably only 7 to 10 days). Female moths deposit 450 to 600 spherical, creamy-white small (0.3 mm) eggs during their life span.

Larvae may be collected by hand, but populations dense enough to make this worthwhile are usually difficult to locate. The best method for collection is probably the removal of infested plants. If high enough numbers are observed, adults may be collected using a sweep net. Waterhyacinth moth caterpillars (larvae) feed within the petiole and on leaf buds. Mature larvae seek out large waterhyacinth leaf petioles and burrow inside, where they excavate a cavity in the middle of the petiole, form a cocoon, and pupate. Such internal feeding leaves a very characteristic curling and browning of the affected leaf, giving the leaf the appearance of a drooping flag. An open exit tunnel is left, permitting the adult (which lacks chewing mouthparts) to escape from the petiole upon completion of pupation.

The waterhyacinth moth is the only introduced agent, other than the two waterhyacinth weevils, that has the capacity to overcome the primary defensive strategy of waterhyacinth. Waterhyacinth moth caterpillars

impact the plants by boring into the bases of leaf petioles and damaging the developing leaves or meristematic tissues (leaf buds). Feeding by caterpillars can cause the entire petiole to break and die. Larval damage is generally restricted to leaf and petiole boring, which can interrupt the movement of nutrients and water within the plant tissues, causing the leaves to collapse.

Sameodes can tremendously damage waterhyacinth in the field, especially those plants growing in more open water whose petioles are greatly enlarged to enhance buoyancy. This morphotype, i.e., bulbous petioles, is the preferred feeding type for *Sameodes*. Bulbous petioles most often occur in early spring when plants are recovering from winter die-back. In most cases, damage from the feeding action of *Sameodes* is sporadic and, by itself, nonthreatening to the waterhyacinth population. However, taken together with the combined feeding action of the two species of waterhyacinth weevils, *Sameodes* damage can be quite effective.

Hydrellia pakistanae - Asian hydrilla leaf miner

Hydrellia pakistanae is a small leaf-mining fly in the family Ephydridae. It originates in Pakistan and India and was first released in the United States on Lake Patrick, Florida, in 1987. It is very similar in habit and appearance to another introduced ephydrid, *H. balciunasi*, and two native *Hydrellia* (mainly *H. bilobifera* and *H. discursa*) frequently found in association with hydrilla in the southeastern United States.

Adult *H. pakistanae* are small flies about 2 mm in length, that reside almost exclusively on or near hydrilla infestations (Figure 6). Adults can

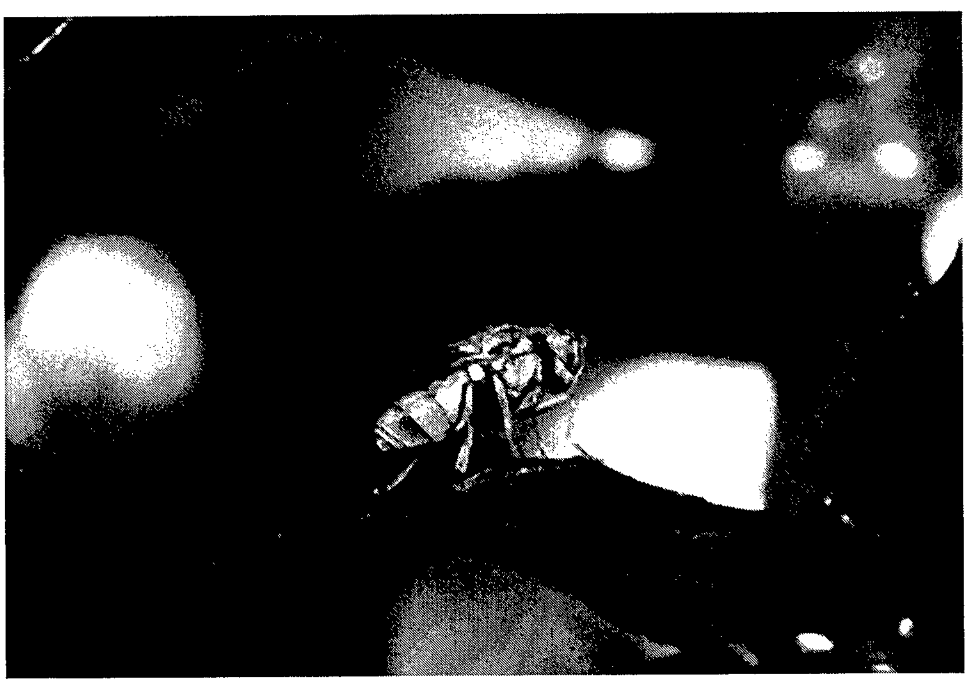

Figure 6. Adult female *Hydrellia pakistanae* on hydrilla leaf

be observed resting on floating hydrilla as well as on other emergent aquatic vegetation in the immediate area of the hydrilla infestation. The flies resemble small gnats that are often seen near ponds and other aquatic systems. They appear to hop along the water surface from one resting place to another instead of actually flying.

Adult *Hydrellia* are relatively difficult to identify in comparison to other species of insect biological control agents. The difficulty arises because of its small size, lack of any obvious distinguishing characters, its similarity to introduced and native *Hydrellia*, and the required use of external reproductive characters for identification. However, with practice and use of a good dissecting microscope, identifications can be made relatively easy.

Adult male *H. pakistanae* can be distinguished from other commonly collected native and introduced species by several characters, including (a) the length of the thorax in comparison to the abdomen length, (b) presence of crossed or cruciate macrochaetae, and (c) shape and size of the macrochaetae.

The abdomen in male *H. pakistanae* is relatively short and is roughly the same size as the thorax (Figure 7).

In contrast, all commonly encountered native male species have abdomens that are one and one-half to two times longer than the thorax (Figure 8).

One should be cognizant that the other introduced *Hydrellia*, *H. balciunasi*, has similar abdomen/thorax configurations as *H. pakistanae*; i.e., the abdomen is roughly the same size as the thorax. The only way to accurately separate the two introduced species is the shape and size of the macrochaetae.

The macrochaetae are small hair-like structures associated with the male external reproductive structures that are thought to be responsible for holding the female in place during copulation. In both species of introduced *Hydrellia*, the macrochaetae are crossed or cruciate (Figure 9). The macrochaetae in *H. pakistanae* are small and more distinctly needle-shaped, while those found in *H. balciunasi* are larger and appear flattened at the tip.

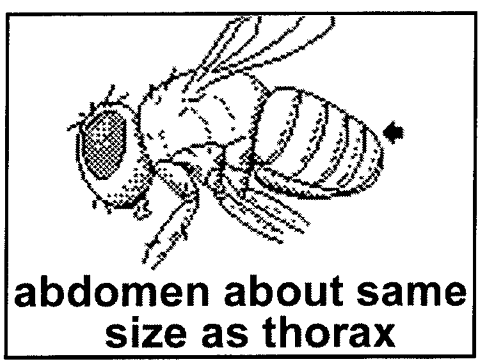

Figure 7. *H. pakistanae* male

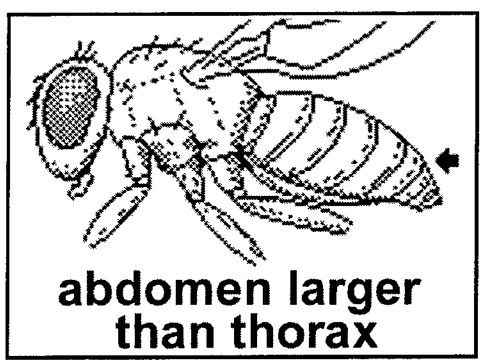

Figure 8. Native male species

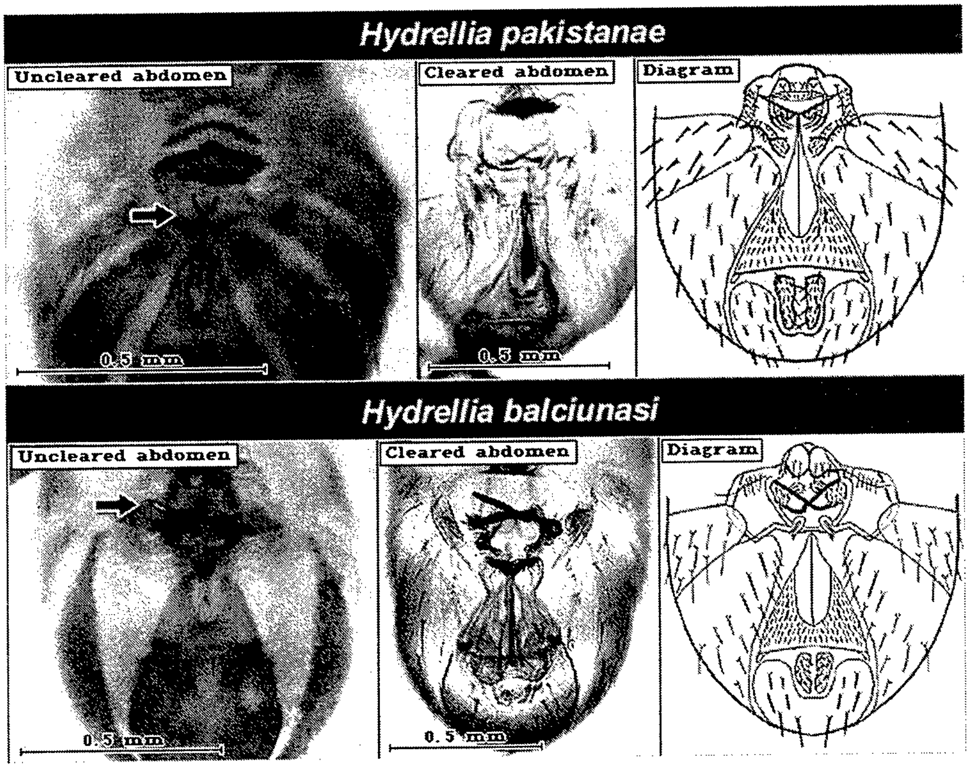

Figure 9. Ventral views of the abdomens' of both *H. pakistanae* and *H. balciunasi* showing the morphology of the male genitalia. Note the cruciate or crossed macrochaetae in both species, which are not found in any native *Hydrellia* species. The primary difference between the two introduced species is the size and shape of the macrochaetae. In *H. pakistanae*, the macrochaetae are smaller and needle-like in comparison to *H. balciunasi* where the macrochaetae are larger and spoon shaped at the end

Female *Hydrellia* are distinguished from native and other introduced *Hydrellia* by the morphology of the genitalia. In females the shape of the cerci is most important. The cerci are hooked or L-shaped in *H. pakistanae* as compared to arrow- or diamond-shaped in *H. balciunasi* (Figure 10).

Eggs are laid on just about any emergent aquatic vegetation including hydrilla and areas near hydrilla infestations. Females lay eggs singly, and each female can oviposit up to several hundred eggs for the length of her reproductive period. Eggs hatch in 3 to 4 days depending on temperature. When the larvae emerge from the eggs, they enter the water in search of hydrilla. Larvae tunnel or mine hydrilla leaves, feeding and destroying about 9 to 12 leaves during the three larval stages. Late, third-instar larvae pierce the stem tissues with two needle-like projections and subsequently pupate. It is believed that piercing the stem allows the pupae to obtain oxygen. Pupae are housed within a protective case known as the puparium, formed from the hardened last larval cuticle. The pupae are roughly cigar-shaped and can be easily confused with axillary buds. The pupal stage lasts from 6 to 15 days, after which the emerging adult floats to the surface in an air bubble.

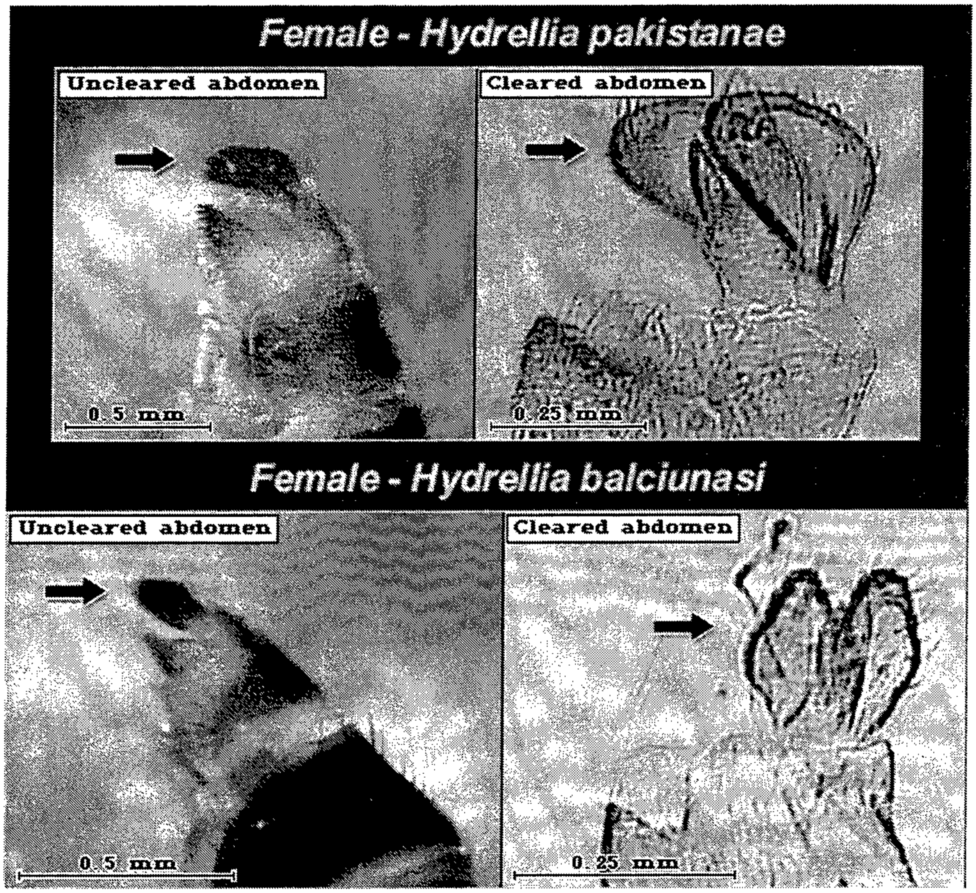

Figure 10. The cerci, located at the posterior end of the abdomen, are used to identify female Hydrellia. In H. pakistanae the cerci are distinctly L-shaped in contrast to H. balciunasi where the cerci are roughly triangular in shape

The best technique for collecting adults is by vacuuming them from the water surface using specially designed hand-held vacuums. The adults can either be released directly into new sites or placed into large containers to allow them to deposit eggs on partially submersed hydrilla. Larvae can also be collected from infested plant material using a Berlese funnel or infested plant material can be harvested and moved to new locations.

Hydrellia pakistanae larvae feed on the internal leaf tissues, leaving distinct tunnels between the leaf surfaces. After larvae feed on a leaf, the leaf appears almost entirely clear, with only limited amounts of green tissue remaining near the margins. The tunneling of hydrilla larvae should not be confused with typical hydrilla leaf clearing, known as bleaching or solarization, which occurs commonly with hydrilla during the summer months. In the case of bleaching, the clearing begins at the distal leaf tips and proceeds to where the leaf attaches to the stem. In extreme cases of bleaching, entire sections of the stem will contain leaves that are entirely clear.

From a distance, a hydrilla mat containing large amounts of *H. pakistanae* feeding appears browned, but upon closer examination, one can observe distinct areas along the stem where feeding has occurred. Overall damage to hydrilla is probably the result of a reduction in total photosynthetic area caused by the leaf damage, thereby reducing growth and vigor and leading to a decrease in the competitiveness of the hydrilla. In addition, some evidence suggests that such feeding reduces the buoyancy of the plant. Limited field observations suggest that larval feeding may predispose the plant to various fungi and other potentially pathogenic attacks.

Hydrellia pakistanae has proven to be successful in damaging and impacting infestations of hydrilla. Established populations occur throughout Florida, north to Muscle Shoals, AL, and west to Austin, TX. Populations of this species have reached high enough levels that > 60 percent of the leaves were damaged. When damaged leaves exceed 25 to 35 percent, large holes typically develop in the mat and, subsequently, portions of the mat sink. At sites in Muscle Shoals, AL, high amounts of damage over several growing seasons have apparently limited the regrowth of the hydrilla in subsequent years.

Survey Summarization

Materials and methods

During the early part of September 1999, nine sites on and adjacent to LRGV Irrigation Districts on the Rio Grande were examined for inclusion in a detailed survey of waterhyacinth and hydrilla infestations and associated biological control agents. Beginning on 27 September 1999, five of the original nine sites were sampled for insect biocontrol agents of waterhyacinth and/or hydrilla. Only five sites were selected because plant levels at four of the nine sites were minimal or nonexistent at the time of the surveys. In addition, plant infestation levels on a per m^2 basis were determined for waterhyacinth, while visual estimates only were accomplished for hydrilla. Sampling methods were similar to those described in the initial proposal but are summarized below for clarification.

Waterhyacinth

Waterhyacinth is the easier of the two plant species to sample for plant biomass and insect agent numbers. It is a floating plant; therefore, access to the infestations can be accomplished relatively easily. Four ¼-m^2 plant samples were randomly selected at each waterhyacinth site. All plants within the ¼-m^2 frame were placed in plastic bags and processed onsite within 48 hr after collection. For each ¼-m^2 sample, plant height, number of rooted plants, above- and below-water surface plant biomass, dead plant weight, number of living/dead leaves, and total number of both adults and

immatures were quantified. As a result of the relatively constant moisture level of the waterhyacinth, plant information is provided as wet plant weight on a per m^2 basis.

Hydrilla

Hydrilla is substantially harder to sample for both plant biomass and insect biocontrol agents. Hydrilla is a submersed plant; therefore, quantification of plant biomass can be accomplished accurately only through the use of Scuba divers who can access the entire submersed portion of the plant within a specific area. In addition, biomass sampling in this manner is very destructive which can significantly add error into future biomass determinations. Hence, estimating hydrilla population levels consisted mainly of examining the infestation visually for the entire site and reporting it as a percent coverage value. In addition, photographs of the site were taken for later comparison.

Hydrellia spp. sampling consisted of several different methods, all of which will be combined to determine insect levels. First, adult *Hydrellia* spp. were hand collected using a Styrofoam® sheet which was pushed in front of a moving boat. As adult *Hydrellia* spp. moved onto the sheet from the hydrilla mat, they were collected using small scintillation vials. Adult collections were used to verify occurrence of each species, since no accurate methods exist for determining species from larval or pupal stages. Number of immatures and percentage leaf damage were quantified from 25 stem pieces (each about 15 to 20 cm in length) collected at random from within the surface canopy of the site. For each site, three subsamples of 25 stem pieces were used to determine means. Individual stems were examined microscopically for damage and presence of immature. From the stem pieces, total number of leaves was estimated from the number of whorls (number of leaves = number of whorls * five), which was then used to calculate the percentage of damaged leaves. Number of immatures was recorded on a per wet-plant-weight basis. In addition, approximately 1 kg of plant biomass was collected and placed into Berlese funnels to extract the mobile immature stages. Number of immatures was recorded on a wet-plant-weight basis for the Berlese funnel extractions. Leaf hardness and nutritional analysis of the collected hydrilla will be ascertained from selected sites as part of another ongoing project funded by the Aquatic Plant Control Research Program (APCRP). Nutritional analysis has not yet been completed and, hence, will not be included in this report.

Site descriptions

As indicated previously, nine sites were selected along the length of the Rio Grande based on proximity to participating irrigation districts, site access, and plant infestation level. Site locations ranged from about 16,090 m (10 miles) west of McAllen, TX, to the western border of Brownsville, TX (Figure 11).

These sites included two irrigation canals and the river inlet within the Hidalgo County Irrigation Water District No. 1 (Figure 12) and the river inlet (Figure 13) and a section of the irrigation canal within Cameron County Irrigation District No. 6 (Figures 14 and 15). The main canals leading from the river to the irrigation district's pumping station were also sampled for the following irrigation districts - La Feria (Figure 16), Harlingen (Figure 17), and Cameron County No. 2 (Figure 18). Plants located

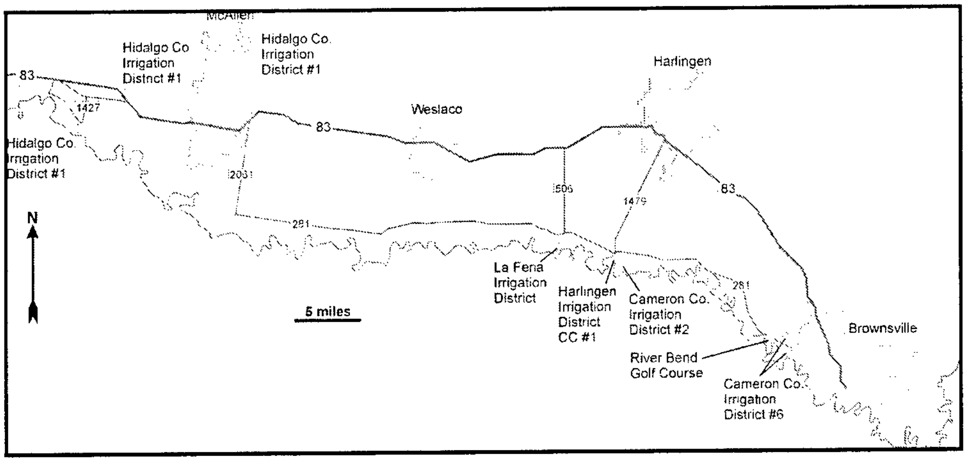

Figure 11. Selected sampling sites for the Rio Grande

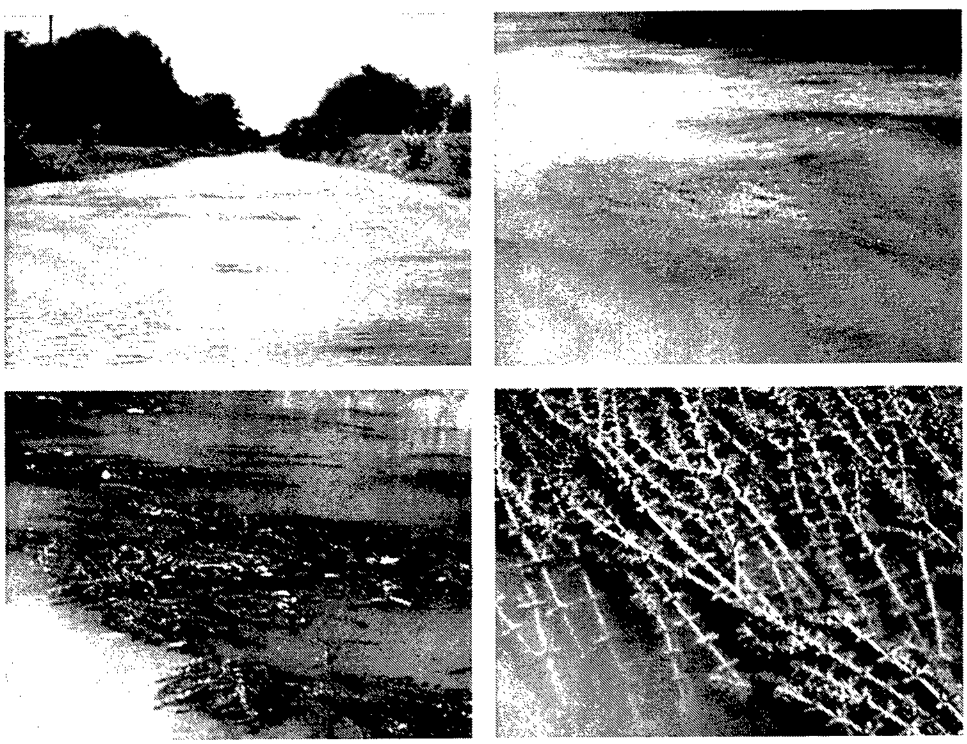

Figure 12. One of the main canals of the Hidalgo County Irrigation Water District No. 1 near Mission, TX. Hydrilla at this site was found scattered throughout the canal system in small clumps. Hydrilla appeared very healthy with long stems that trailed on the surface with the water flow. Hydrilla in some areas appeared as larger infestations where the individual clumps had begun growing together

adjacent to the River Bend Golf Course (Figure 19) were also sampled. Table 1 provides more detailed information on each site and the plant species sampled.

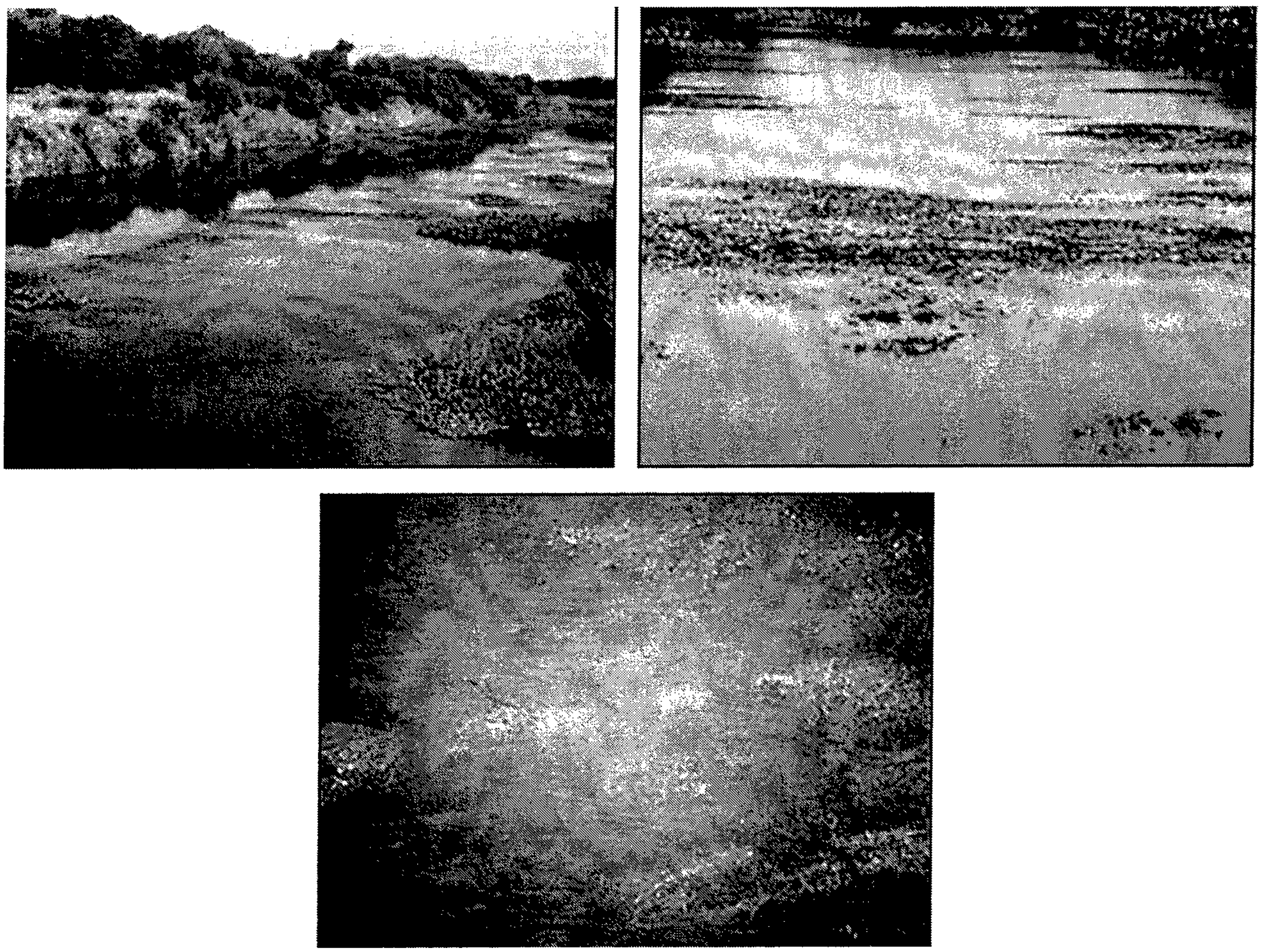

Figure 13. Series of views of the Cameron County Irrigation District No. 6 Pumping Station located directly on the Rio Grande. Waterhyacinth was present in distinct clumps, but the infestation never extended across the entire river (top left). While some feeding damage by *Neochetina* spp. was observed, it was mostly minimal throughout the site. This site, located near a weir, had increased water flows in the general area. This may have precluded the accumulation of waterhyacinth except directly on the rocks composing the weir, which tended to trap and hold the waterhyacinth. Hydrilla infestations were large at this area and tended to completely cover this section of the river (top left, top right). While the hydrilla appeared healthy, there were areas where native species were encroaching into the mat. For example, the lower photograph shows significant coverage of *Heteranthera dubia* (water star grass) encroaching into the hydrilla infestation

Figure 14. Canal leading away from the Cameron County Irrigation District No. 6 Pumping Station. Note the large waterhyacinth infestation completely covering the canal. While some areas of the canal were open, a majority was completely covered. The waterhyacinth at this site was very healthy with only minimal damage from biological control agents. Limited encroachment by other plant species was observed at this site

Figure 15. Close-up view of canal at Cameron County Irrigation District No. 6. Note healthy plants with only limited feeding damage by *Neochetina* spp.

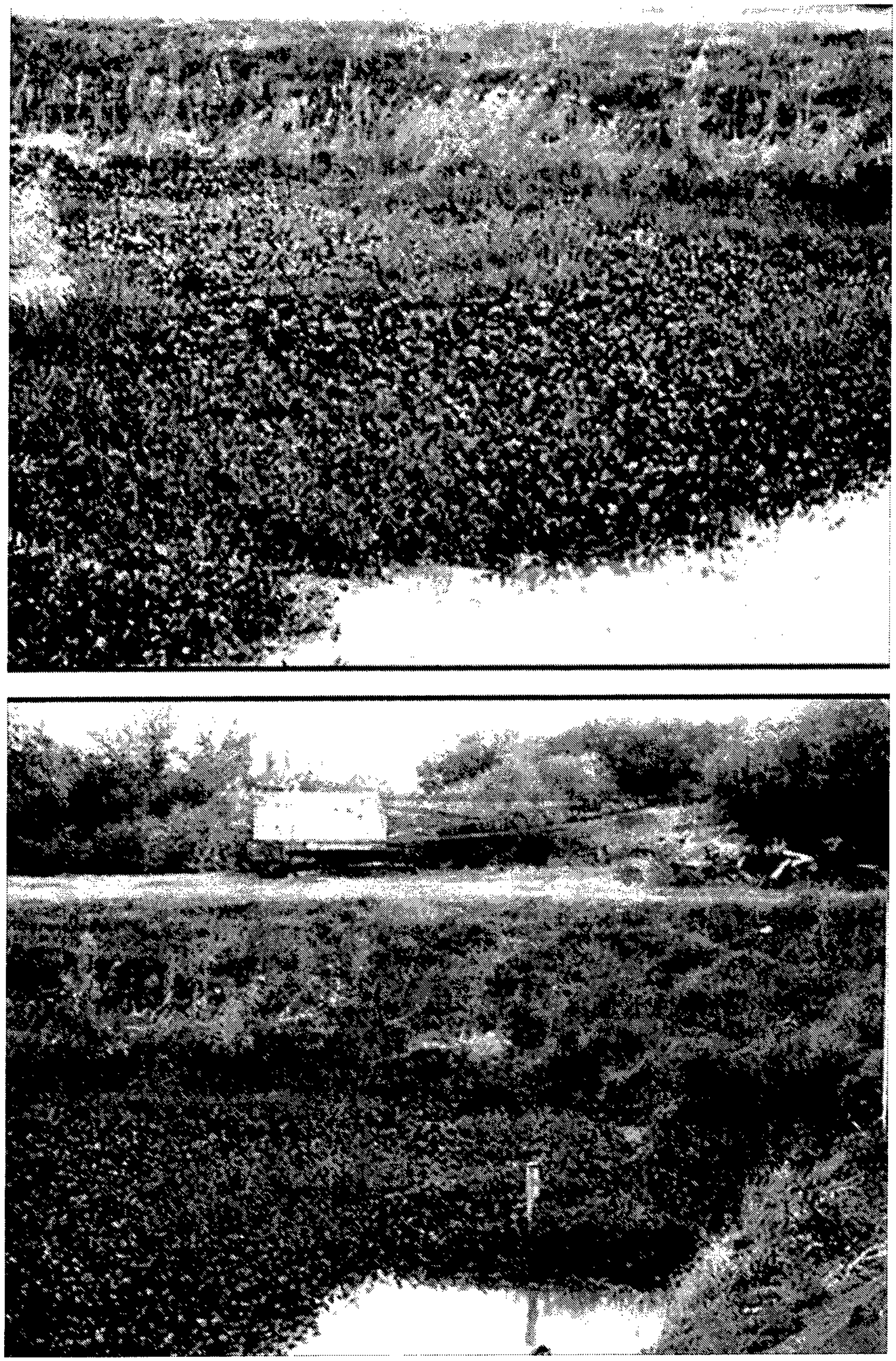

Figure 16. Composite view of the La Feria Irrigation District. These photographs were taken on the canal leading from the Rio Grande to pumping station. Note the extensive infestation, which is apparently stressed by the feeding action of *Neochetina* spp. as indicated by the browning and curling leaves and the presence of other plant species encroaching into the infestation

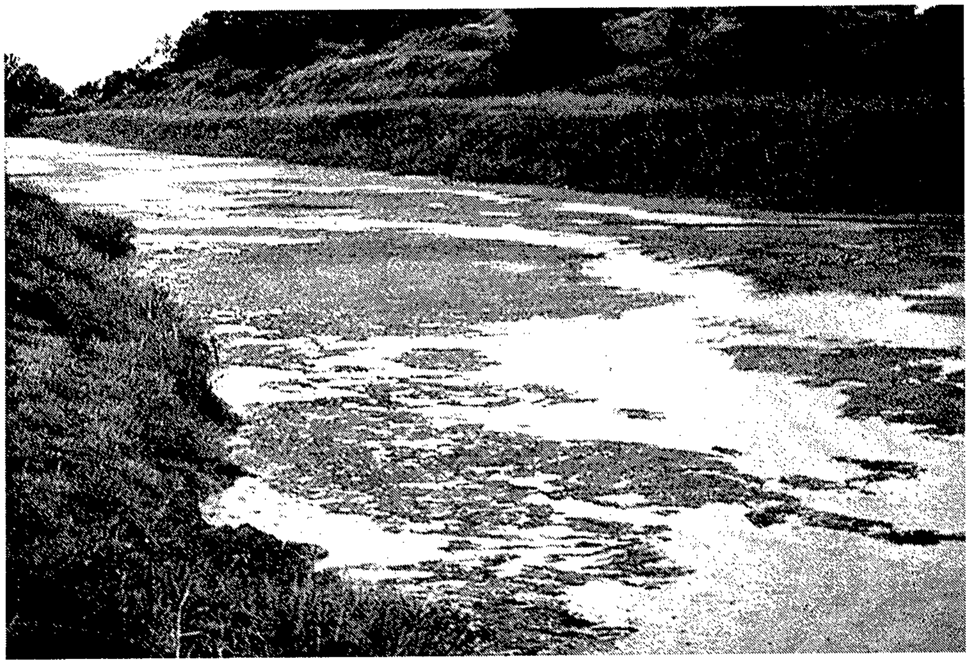

Figure 17. Canal leading from the Rio Grande to the Harlingen Irrigation District (Cameron County) No. 1 Pumping Station. Minimal waterhyacinth was present at this site and most of the plants appeared stressed. While an extensive hydrilla infestation appears present, closer inspection revealed it was mainly algae covering what had probably been an infestation. Reasons for the hydrilla disappearance are unknown

Figure 18. Canal leading from the Rio Grande to the pumping station of Cameron County Irrigation District No. 2. Similar to the Harlingen Irrigation District Cameron County No. 1 Canal (Figure 17), the waterhyacinth was highly stressed and the hydrilla infestation was minimal and mainly covered with copious quantities of algae

Figure 19. Composite view of an extensive waterhyacinth infestation on the Rio Grande adjacent to the River Bend Golf Course. While the waterhyacinth completely choked the river at this point (top), the plants appeared highly stressed by the feeding action of the insects and in numerous areas large populations of other plant species were observed encroaching into the mat (top, bottom). The plant species most commonly seen within the mat was another introduced species, *Arundo donax* (giant reed), which was commonly observed along large stretches of bank

Table 1.
Detailed Descriptions of Survey Sites Including Information on Site Location, Physical Description of Site, Plant Status, and Plants Sampled

Site Type	Site Name	Site Description	Plant Species Present
Irrigation Canals	Hidalgo County Irrigation District No. 1 (Figure 12)	Small irrigation canal located off McColl Rd. between F. Gonzalez and Hobbs. Water flow rates vary because of water demands.	Minimal levels of hydrilla mainly in small clumps throughout the canal. More extensive infestations were found in some areas.
	Hidalgo County Irrigation District No. 1	Small irrigation canal. Water flow rates vary because of water demands on the system.	No hydrilla or waterhyacinth present. Small populations of water star grass present in some areas. Site not sampled.
	Cameron County Irrigation District No. 6 Canal Site (Figures 14 and 15)	Small irrigation canal leading away from the actual pumping station. Minimal flows were detected in the canal but could increase during those times when water is being utilized at higher rates.	Large sections of canal were entirely covered with waterhyacinth. Visual inspection revealed limited feeding damage by insect biocontrol agents. Flowering commonly observed.
Inlets to Pumping Station	Hidalgo County Irrigation District No. 1	Small inlet canal leading off of the main river channel to pumping station. Water flows apparently vary because of water demands.	No hydrilla or waterhyacinth present. From a reasonable distance site appears to have large quantity of submersed vegetation based on the presence of copious amounts of algae covering the inlets edges. However, no submersed vegetation could be located. Site not sampled.
	La Feria Irrigation District (Figure 16)	Terminating area of canal where water is pumped from the river to irrigation canal network. This site is not directly on the river. Only minimal water flow detected. Water flows could vary based on demand.	Large infestations of waterhyacinth present covering a majority of the site. Infestation was not entirely monotypic as other plant species (e.g., *Arundo donax*) were encroaching into the mat. Some mechanical removal of waterhyacinth was observed. No hydrilla was present.
	Harlingen Irrigation District Cameron County No. 1 (Figure 17)	Terminating area of canal where water is pumped from the river irrigation canal network. This site is not directly on the river. Only minimal water flow detected.	Minimal levels of waterhyacinth present mainly confined to the shoreline. Most of the plants appeared stressed as evidenced by large sections of the mat having plants of a light green to yellow color. Hydrilla was also present at this site and from a distance a majority of the site appeared to be covered. However, the hydrilla at this site was highly stressed with large amounts of algae. The hydrilla under the alga was browned, losing leaves, and in various states of decomposition. No plants of either species were collected at this site because of their poor condition. Adult *Hydrellia* were hand collected to determine presence or absence of the hydrilla biocontrol agents.
	Cameron County Irrigation District No. 2 (Figure 18)	Terminating area of canal where water is pumped from the river to irrigation canal network. This site is not directly on the river.	Minimal levels of waterhyacinth present. Most appeared stressed as evidenced by large sections of the mat having plants which were yellow and appeared to be dying. Hydrilla was also present and appeared to cover a large portion of the site; however, it was highly and mostly covered with copious amounts of algae. The hydrilla under the alga was browned, losing leaves and in various states of decomposition. In some cases, the hydrilla was still green, but leaves were readily shed once the plants were removed from the water. Only one sample of hydrilla was collected at this site because of the poor condition of the plants.
Sites Directly on River	Cameron County Irrigation District No. 6 River Site (Figure 13)	Unlike the other irrigation district sites, the pumping station at this location was located directly on the river. The water uptake pipes were upstream of a weir system, which produced higher than normal flows in this vicinity of the river.	Both hydrilla and waterhyacinth were present throughout this site. The waterhyacinth did not form an extensive infestation across the river and instead was located in clusters along the bank. The plants were tall and in excellent condition exhibiting only minimal stress. Hydrilla was present in large infestations immediately upstream of the weir and in front of the pumping station. The mat was very thick and extensive and continued throughout the entire stretch of the river. The plants were very dense with long stems that trailed in the water with the current.
	River Bend Golf Course (Figure 19)	Site was located directly on river adjacent to golf course. Mechanical control of waterhyacinth and hydrilla had been accomplished at this site in prior years.	Large infestation of waterhyacinth was present and completely choked the river. Plants appeared highly stressed with limited flowering. Encroachment into the mat by other plant species, mainly *Arundo donax*, was observed.

Survey results

The following is a summary of the survey accomplished during September 1999 for both waterhyacinth and hydrilla and associated insect biocontrol agents on and adjacent to LRGV Irrigation Districts on the Rio Grande. The information is divided into two major sections by plant species.

Waterhyacinth. Biomass of waterhyacinth samples varied significantly from site to site (Figure 20). Highest total biomass (i.e., above, below, and dead biomass per m^2 combined) was found at the River Bend Golf Course site (20.67 kg/m^2) with the lowest observed at Cameron No. 6 River site (2.74 kg/m^2). Total biomass differences in this case were almost eight-fold higher for the River Bend Golf Course site relative to that found at the Cameron No. 6 River site. Examining each of the separate biomass partitions (i.e., above water, below water, and dead material) reveal that at most sites quantity of each partition on a per m^2 is similar. The only exceptions occurred at the River Bend Golf Course site where significantly higher biomass occurred for all partitions relative to the other sites. In addition, significantly lower above-water biomass occurred for the Cameron No. 6 River site, as well.

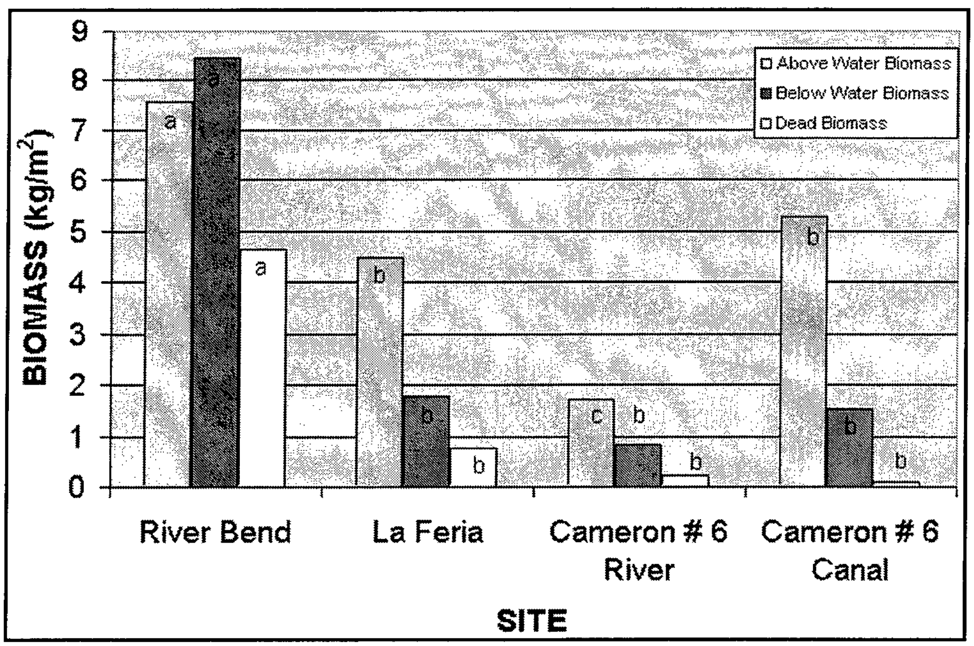

Figure 20. Total amount of above, below, and dead biomass per m^2 at each of the four sites surveyed for waterhyacinth on and adjacent to the Rio Grande. Means which are significantly different at $p < 0.05$ are marked by different letters within a biomass partition (above water biomass – df = 3, 11, $F = 12.58$, $p = 0.0007$; below water biomass – df = 3, 11, $F = 7.35$, $p = 0.0056$, and dead biomass – df = 3, 11, $F = 9.94$, $p = 0.0018$)

Differences were also observed in the percentage of each biomass partition relative to the total biomass present at a site (Figure 21). At three of the four sites, the highest percentage of biomass was found in the plant material located above the waterline, i.e., mainly leaves, petioles, and ligules. Percentage of total biomass that was accounted for by the above-water biomass partition ranged from 75 percent at the Cameron Irrigation District No. 6 Canal site to 50 percent for the Cameron Irrigation District No. 6 River site. The next highest partition was the below-water biomass, which included roots, stolons, and stems. This ranged from about 35 percent (Cameron Irrigation District No. 6 River site) to 20 percent (Cameron Irrigation District No. 6 Canal site). In all cases, the smallest percentage of biomass was accounted for by the dead biomass, which ranged from about 2 percent (Cameron Irrigation District No. 6 Canal site) to 10 percent (Cameron Irrigation District No. 6 River site). However, at the River Bend Golf Course site, above-water and below-water biomass was essentially the same (about 37 percent) and total percentage of dead biomass appeared higher relative to the other three sites (about 25 percent).

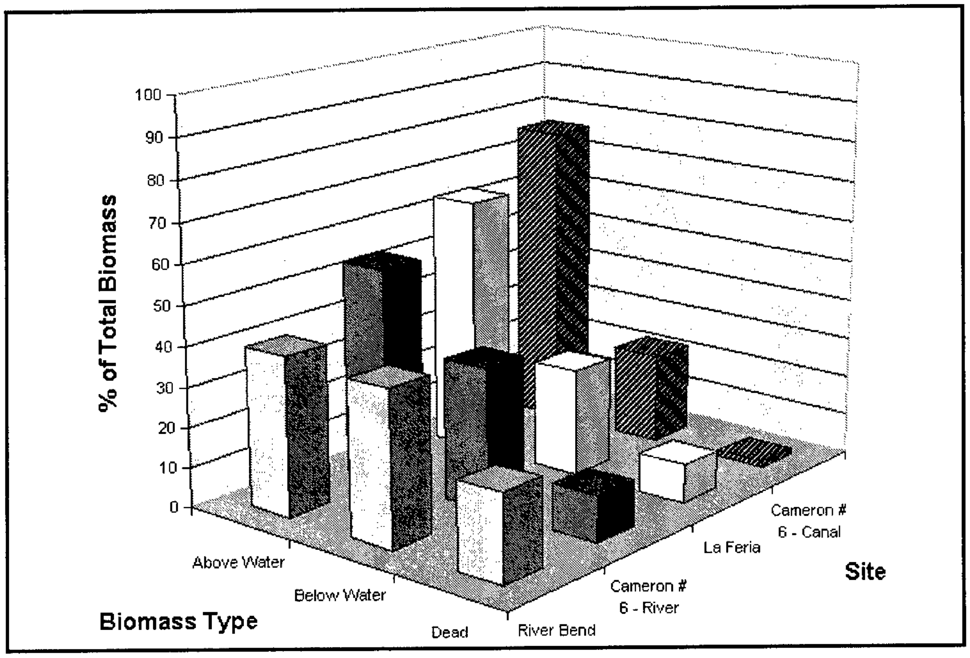

Figure 21. Percentages of each biomass partition for the four sites sampled for waterhyacinth on and adjacent to the Rio Grande

Other plant parameters were similar for each site and included plant height, plant number (Figure 22), leaves per plant, and number of flowers per m^2 (Figure 23). Of these four plant parameters, significant differences were observed for leaves per plant only. Plant height ranged from 40 cm at the Cameron Irrigation District No. 6 River site to a high of about 60 cm at the Cameron Irrigation District No. 6 Canal site. Plant number per m^2 was also similar across sites and ranged from 40 plants/m^2 at the Cameron Irrigation District No. 6 Canal site, to 96 m^2 at the River Bend Golf Course site. Repeating this sampling with higher sample sizes may have revealed the presence of more significant differences.

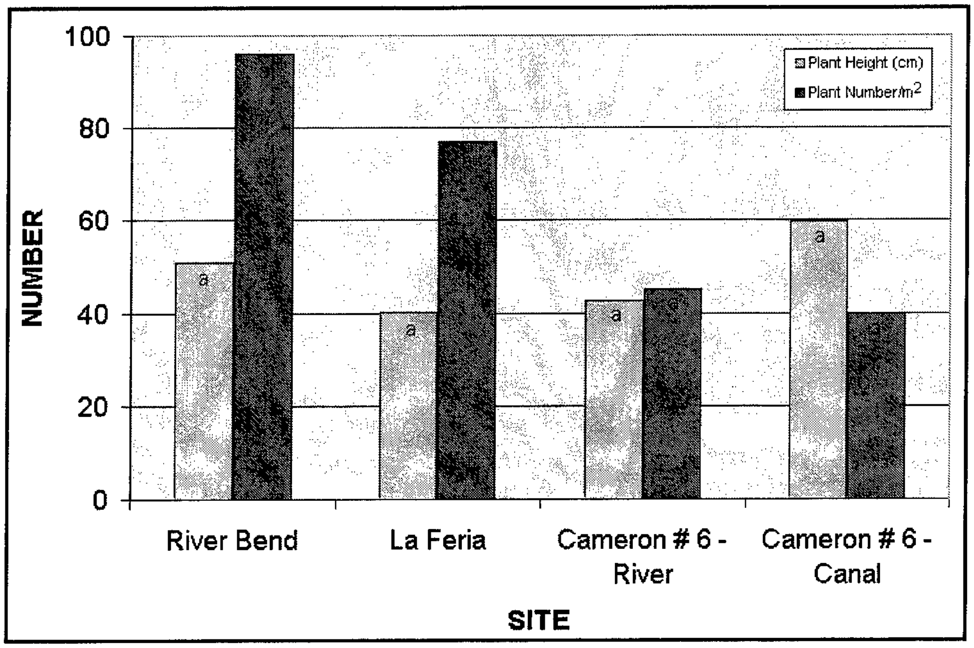

Figure 22. Plant height (cm) and number of plants per m^2 for waterhyacinth sampled at four sites on and adjacent to the Rio Grande. Means for each parameter followed by different letters indicate significant differences at $p < 0.05$ level (Plant height – $F = 2.16$, $df = 3, 11$, $p = 0.1507$, Plant number – $F = 2.82$, $df = 3, 11$, $p = 0.0880$)

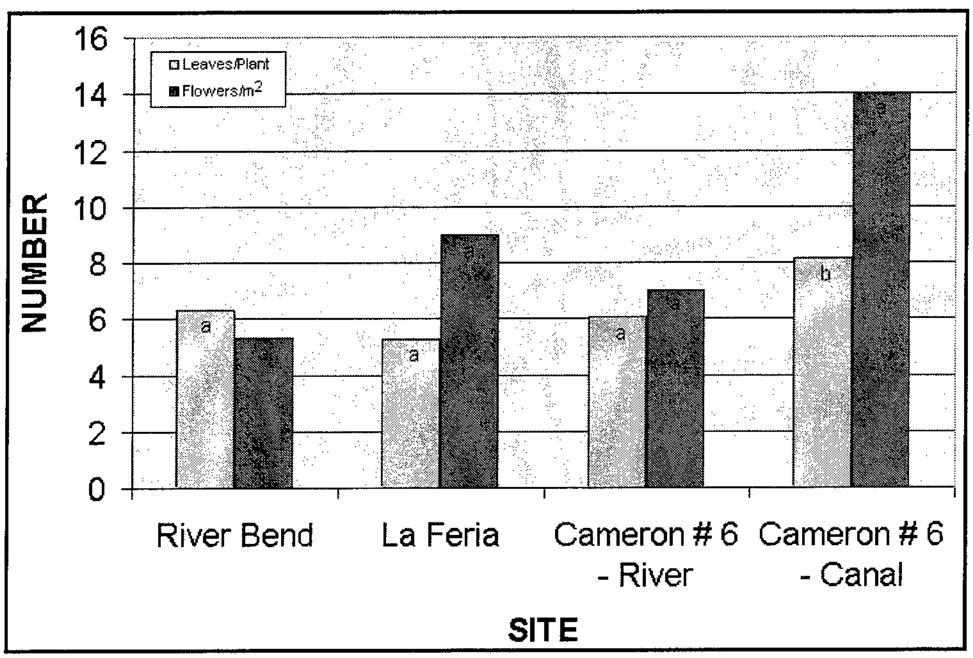

Figure 23. Leaves per plant and flower stalks per m^2 for waterhyacinth sampled at sites on and adjacent to the Rio Grande. Means followed by different letters indicate significant differences at the $p < 0.05$ level (Leaves/plant – $F = 10.24$, $df = 3, 11$, $p = 0.0016$, Flowers/m^2 – $F = 0.67$, $df = 3, 11$, $p = 0.5860$)

Flowers per m^2 ranged from five flowers per m^2 (River Bend Golf Course) to 14 flowers per m^2 (Cameron Irrigation District No. 6 Canal site; Figure 23). The only statistical differences were detected in the number of living leaves per plant where the highest number was observed at the Cameron Irrigation District No. 6 Canal site, which had over eight leaves per plant.

Two species of introduced biocontrol agents of waterhyacinth were also commonly observed at most of the sites sampled. The insect agents included *N. eichhorniae* and *N. bruchi,* the mottled and chevroned water-hyacinth weevils, respectively. Numbers of each of the species were statistically similar but over two-fold higher means were observed for *N. bruchi* (11.7/m^2; Figure 24). Statistical differences in the number of each species may be detected by taking a larger number of replicates at each site. Finding such high numbers of *N. bruchi* was unusual as most waterhyacinth sites sampled in Texas typically contain only *N. eichhorniae,* with only limited numbers of *N. bruchi* (personal observations). No *S. albiguttalis* were observed. *Sameodes albiguttalis* was probably present as well but is typically found in higher numbers during the early spring regrowth period when the plants are growing more laterally to fill up sites opened during the winter die-back period. Plants in this stage of growth typically have enlarged petioles for floatation, which is a more ideal larval feeding site for *S. albiguttalis* than plants without enlarged petioles.

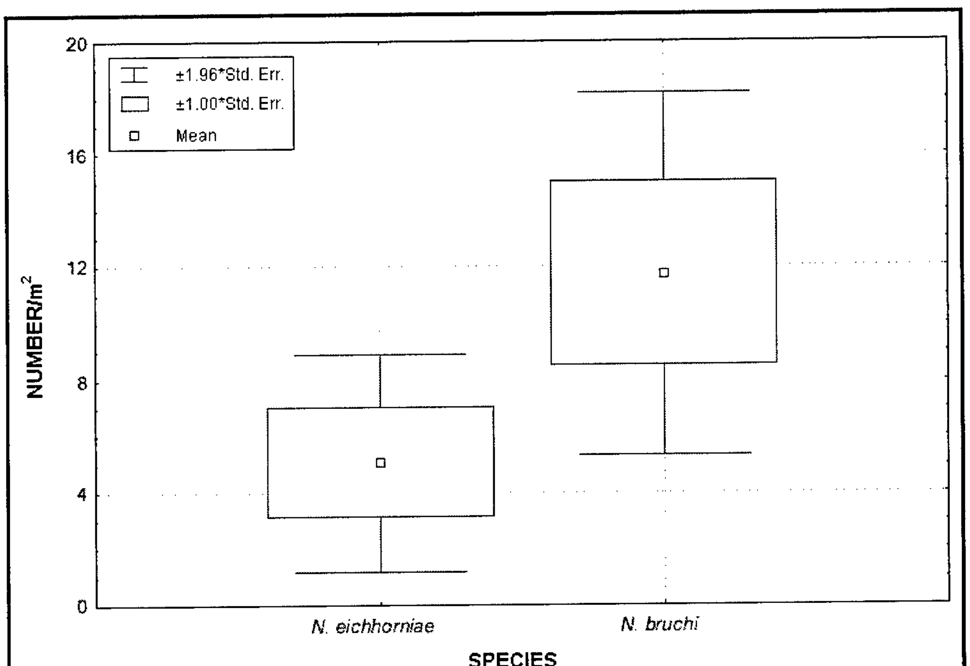

Figure 24. Mean number of each species of *Neochetina* spp. per m^2 collected from sites on and adjacent to the Rio Grande. No significant differences were detected ($F = 3.04$, $df = 1, 28$, $p < 0.0922$). Please note, however, means were higher for *N. bruchi* and the p level is less than 0.10

Of the four sites sampled, total number of all life stages of both *N. eichhorniae* and *N. bruchi* differed considerably from site to site (Figure 25). For example, highest numbers were observed at the River Bend Golf Course site with 80 individuals/m^2. This was followed by the La Feria site (ca. 60 individuals/m^2), Cameron No. 6 River site (36 individuals/m^2), with the lowest amount observed at the Cameron No. 6 Canal site (19 individuals/m^2; Figure 25). Numbers of adult and larval stages also varied from site to site. Highest numbers for both stages were observed for the River Bend Golf Course site; the lowest, at the Cameron No. 6 Canal site.

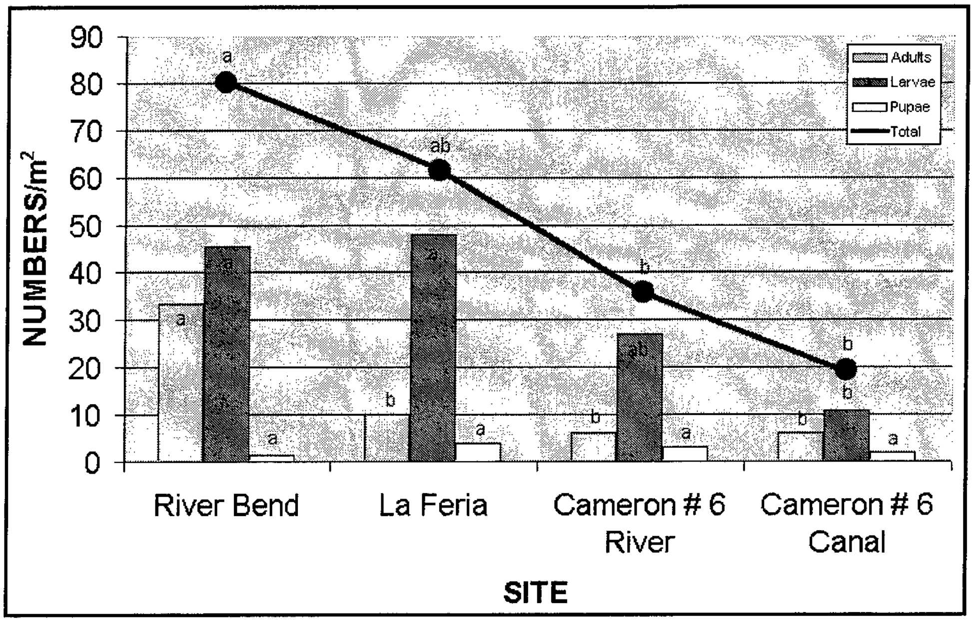

Figure 25. Total number of all life stages of *Neochetina* spp. and number of adults, larvae, and pupae on a per m^2 basis for waterhyacinth sites on and adjacent to the Rio Grande. Means followed by different letters are significantly different at $p < 0.05$ level (Total/m^2 – $F = 5.80$, df = 3, 11, $p = 0.0126$, Adults/m^2 – $F = 4.16$, df = 3, 11, $p = 0.0338$, Larvae/m^2 = 5.67, df = 3, 11, $p = 0.0135$, Pupae/m^2 – $F = 0.32$, df = 3, 11, $p = 0.8090$)

Strong relationships between various plant parameters and number of insect agents were observed for several variables (Figure 26). While more data should be collected to verify these trends, the current data serve as an illustration of the impact *Neochetina* spp. appears to be having on waterhyacinth infestations in the Lower Rio Grande area. For example, strong negative correlations exist between the average number of adults and larvae and number of larvae/m^2 with the number of flower stalks/m^2. At sites with only 20 total *Neochetina* spp./m^2, the number of flower stalks/m^2 averaged about 14 individuals/m^2. This can be contrasted to sites with 80 *Neochetina* spp./ m^2 where the number of flower stalks/m^2 was reduced over two fold. Similarly, higher number of larvae appeared to impact the flowering potential of the waterhyacinth. Sites with > 45 *Neochetina* larvae/m^2 exhibited > two-fold reductions in number of flower stalks/m^2 in comparison to sites with only 5 larvae/m^2. In addition, increases in the amount of dead plant biomass/m^2 were associated with higher numbers of adults/m^2,

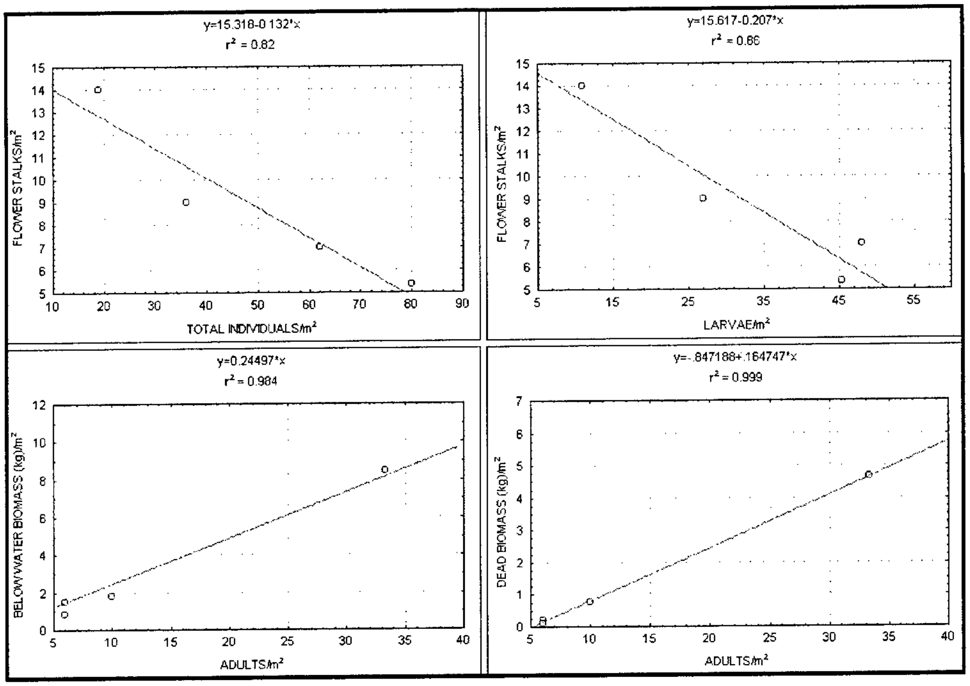

Figure 26. Relationships between various insect parameters and plant characteristics. Points used in the analysis are the means taken from each site with n = 3 or n = 4 depending on the site (flowers/m^2 vs. total individuals/m^2 – $F = 18.08$, df = 1, 2, $p = 0.0511$; flowers/m^2 vs. larvae/m^2 – $F = 18.95$, df = 1, 2, $p = 0.0489$; below water biomass/m^2 vs. adults/m^2 – $F = 247.36$, df = 1, 3, $p = 0.0006$; dead biomass/m^2 vs. adults/m^2 – $F = 3487.3$, df = 1, 2, $p = 0.0003$)

and higher numbers of adults were also shown to be positively related to the below-water biomass partition.

Waterhyacinth in the irrigation districts along the Rio Grande showed large variation in the amount and degree of damage inflicted by the introduced insect biological control agents. Sites such as the River Bend Golf Course and the La Feria Irrigation District not only had higher number of *Neochetina* spp. of all life stages, there were signs that insect impact was stressing the plants to some degree. This is evidenced by decreased flowering, significantly lower number of leaves per plant, and increases in dead plant material at these sites. In addition, the encroachment of other plant species into the waterhyacinth infestation was observed at both the River Bend Golf Course and La Feria sites; another indication that insect feeding damage was stressing the plants and allowing other, normally noncompetitive, plants an advantage.

However, plant biomass observed at both the River Bend Golf Course and La Feria Sites was among the highest observed. The fact that these sites are apparently older infestations may account for the higher biomass. These sites may also exhibit differences in nutritional content, which has been shown to influence insect population growth and reproductive capability (Center and Van 1989). In addition, based on past observations at irrigation district sites along the Rio Grande, insect damage has just recently

reached observable stress levels. Small qualitative surveys conducted during late summer 1998 indicated that insect biological control agents were at minimal levels at all of the river sites examined. Most sites examined had less than one insect per plant with only limited visible signs of insect feeding. The status of the plants in 1998 was very similar to those found at the Cameron Irrigation District No. 6 Canal site during the 1999 survey. While biomass levels were low overall at the Cameron Irrigation District No. 6 Canal site during 1999, the plants were among the healthiest observed and had only minimal signs of feeding damage, the highest mean number of flowering stalks, the tallest plants, and a more typical biomass distribution. Biomass distributions in healthy stands of waterhyacinth are typically higher in the above water partition followed by below water, then dead biomass. However, nutritional input can have a profound effect on how the biomass is distributed (Center and Van 1989).

Insect biological control populations appear to be expanding at some sites in the Lower Rio Grande Valley area since first surveyed during 1998. This trend can be expected to continue in the absence of any adverse environmental effects and/or impact to the insect populations due to chemical applications, increased water flows, or large-scale mechanical control operations. The presence of both species of *Neochetina* is also encouraging since some data suggests higher impact when both are present at the same site.[1] While it is unknown how long the agents have been in the area, based on the minimal surveys conducted during 1998, it appears that populations have just begun to increase.

Based on past releases of the *Neochetina* spp., it usually takes from 3 to 6 years before significant impact, ultimately leading to decreases in waterhyacinth populations is observed (Center, Cofrancesco, and Balciunas 1990). Impact time has been shown to be dependent on the number of insects migrating or released into a specific area. Since several sites only had minimal levels of the insect agents, it may be prudent to begin releasing additional individuals into the area. The release individuals can be collected from nearby Texas-based sites or purchased directly from dealers in the Florida area and released into those sites with low insect population levels. It is important to obtain at least some of the release individuals from Florida sites in an effort to strengthen the genetic base of the insects already established.

Hydrilla. Hydrilla populations appeared to differ significantly from site to site. While no quantitative measurements of the hydrilla populations were made, visual inspections of the sites revealed hydrilla populations ranging from small scattered populations (Hidalgo County Irrigation District No. 1 Main Canal) to extensive infestations across major portions of the river (Cameron County Irrigation District No. 6 River site). Hydrilla, in most areas, appeared healthy with only limited encroachment by other native species, which is an indication of hydrilla stress. The only area

[1] Personal communication, August 1998, Dr. T. D. Center, Ft. Lauderdale, Florida.

where encroachment was seen to any great extent was on the Cameron County Irrigation District No. 6 River site where populations of *Heteranthera dubia* were seen growing intermixed with the hydrilla infestation. At two sites, Harlingen County Irrigation District (Cameron County) No. 1 and Cameron Irrigation District No. 2, the hydrilla was obviously stressed with large amounts of algae completely covering the infestation and only limited hydrilla present beneath the algae. Reasons for the declines at these sites are unknown, but insect damage is probably not a factor since population numbers of the agents were low.

The only insect agent of hydrilla collected from the survey sites was *H. pakistanae*. This even included the Harlingen Irrigation District (Cameron County) No. 1 site where the hydrilla was so stressed that not even a single biomass sample could be collected. At this site, adult *H. pakistanae* were collected in low but consistent levels from the remaining matted hydrilla. In fact, the highest *H. pakistanae* populations observed (ca. 350 immatures/kg with 4 percent of the leaves damaged) occurred at the Cameron County Irrigation District No. 2. This was surprising since the hydrilla was obviously stressed by some unknown factor(s) at this site (Figure 27). While population levels of *H. pakistanae* appeared relatively low, they are definitely established and appear to have spread throughout the entire Lower Rio Grande Valley area.

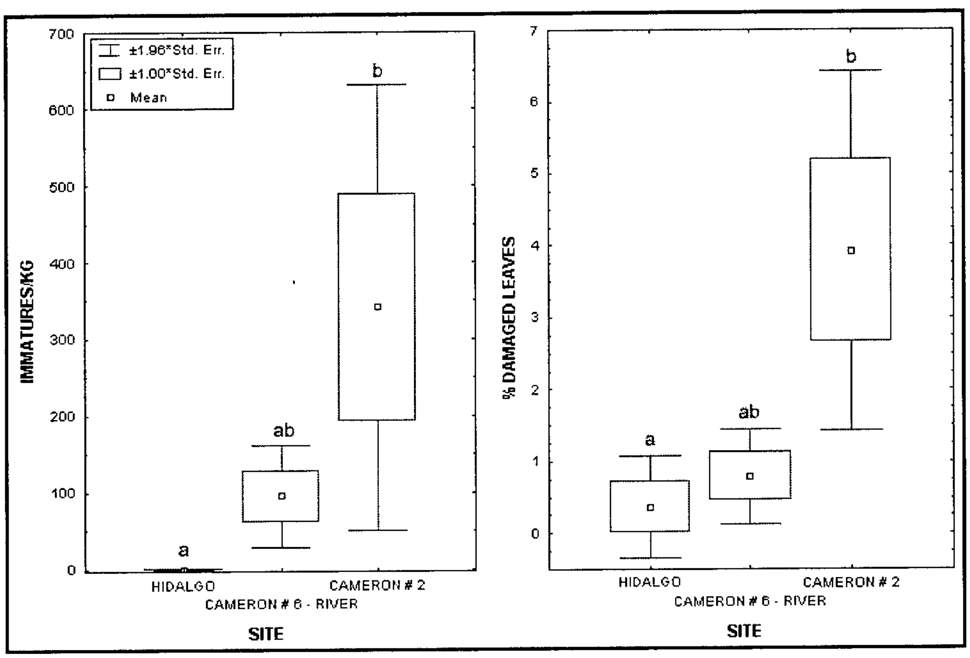

Figure 27. Total number of *H. pakistanae* immatures/kg and percentage of damaged leaves from sites on irrigation districts on or adjacent to the Rio Grande. Information was collected from 25 stems taken at random from each site. Means with different letters indicate significant differences at the $p < 0.05$ level (immatures/kg – $F = 9.61$, $df = 2, 172$, $p = 0.0001$; percentage leaves damaged – $F = 9.15$, $df = 2, 172$, $p = 0.0002$)

It was surprising to collect any *H. pakistanae* from this area, since the closest release site is over 400 km to the north and populations at this site (Choke Canyon Reservoir) have remained low to nonexistent since their release in the early to mid 1990's. Populations of *H. pakistanae* were first discovered in the Lower Rio Grande Valley area during initial surveys of the area in 1998 (Grodowitz et al. 1999). The only hydrilla site surveyed during the 1998 sampling trip was the Cameron County Irrigation District No. 6 River site. At this time, approximately 1.2 percent of the leaves were damaged and immature numbers were about 160 immatures per kg of wet plant material. This is very similar to what was observed during the more extensive 1999 sampling trip where immature numbers averaged about 100 immatures/kg and percentage damaged leaves averaged about 0.9 percent (Figure 27).

It has been known for several years that a pupal parasite of native *Hydrellia* spp. parasitizes the introduced *H. pakistanae.* This pupal parasite, *Trichopria columbiana*, a small diapryid wasp, can have a profound impact on the introduced *Hydrellia* spp. Research is currently underway which is designed to quantify pupal parasite rates under field conditions at sites in Texas, Florida, and Georgia. In the case of the Rio Grande sites, *T. columbiana* was collected from several of the hydrilla sites surveyed during 1999. All sites had low levels of wasp emergence with the Cameron County Irrigation District No. 2 having the highest level with about seven individuals/kilogram as determined from Berlese funnel extractions. No *T. columbiana* were detected at the Cameron County Irrigation District No. 6 River site, and only one *T. columbiana* adult was collected from the four samples collected from the Hidalgo County Irrigation District No. 1 site. It is interesting but not unusual that the highest number of pupal parasites were collected from the site with the highest population level of *H. pakistanae* (i.e., Cameron County Irrigation District No. 2).

It is strongly recommended that additional releases of *H. pakistanae* be accomplished at one or more of the sites along and adjacent to the Rio Grande. Because of the limited numbers of *H. pakistanae* found in the Lower Rio Grande Valley area and the strong possibility that introductions/immigration into the area occurred from a small number of individuals, additional releases would be beneficial. Such introductions would allow the already existing population to increase more rapidly and add diversity to the genetic base.

Steps for Incorporating Insect Biological Control Strategies in Lower Rio Grande Valley Area

With the completion of the initial surveys of both waterhyacinth and hydrilla in the Lower Rio Grande Valley area, the design of a feasible introduction and monitoring program for the use of insect biological control agents is relatively simple and straightforward. Basically, there are five main steps to undertake when designing and incorporating a new insect biocontrol program into an existing aquatic plant management program.

Step 1. The first step has already been accomplished with the completion of the initial surveys along sections of the Lower Rio Grande Valley area. This step allows us to determine the degree of plant infestation and the population sizes and impact caused by the introduced and native insect herbivores. Such surveys are one of the earlier steps toward selecting potential release sites for future introductions.

Step 2. The second step is to conduct a small qualitative survey in spring 2000 to verify that no major changes have occurred in the status of both the plants and their associated insect biocontrol agents. This would entail surveying the same sites examined during the fall 1999 and qualitatively assessing plant infestations and numbers of agents relative to what was observed during the fall 1999.

Step 3. The third step would be to select potential release sites for agents of both waterhyacinth and hydrilla based on the information gathered during the fall 1999 and spring 2000. While some sites were tentatively identified during the fall 1999 survey, other sites may have experienced an increase in plant infestation or were not examined during that time period and may be more ideal for releases during the spring 2000. Potential sites identified during the 1999 survey include the Cameron County Irrigation District No. 6 Canal site for waterhyacinth and the Hidalgo County Irrigation District No. 1 and Cameron County Irrigation District No. 6 River site for hydrilla.

Step 4. Once the sites have been identified for release, the fourth step is to determine the number of agents to be introduced. While no exact method for estimating the numbers of agents for release has been devised, we generally try to release at least 20,000 *Neochetina* spp. and near to 50,000 *H. pakistanae* per area, especially if the infested area is less than 8.09 hectares (20 acres). The more agents released the better the chance for survival, establishment, and eventual impact. In addition, it is a good approach to release high numbers of individuals to increase genetic diversity at the release area. This will afford a greater chance for agent survival as local environmental conditions change.

Step 5. The final step involves developing a simple biseasonal monitoring plan to allow for determination of the success of the releases and ultimate impact on the plant infestations. Initially, monitoring should be accomplished at the end of the growing season after the releases have been made. Subsequent monitoring should be carried out twice during the active growing season; once in the beginning and once at the end of the growing season. Sampling need not be as detailed as that accomplished during the fall 1999, but some type of quantitative sampling should be attempted to determine establishment and population increases of the agents as well as associated impact. In addition, monitoring would aid in minimizing the impact from other types of management techniques on the biocontrol procedures.

References

Center, T. D., and Durden, W. C. (1981). "Release and establishment of *Sameodes albiguttalis* for the biological control of waterhyacinth," *Environmental Entomology* 10, 75-80.

Center, T. D., and Van, T. K. (1989). "Alterations of waterhyacinth (*Eicchornia crassipes* (Mart) Solms) leaf dynamics and phytochemistry by insect damage and plant density," *Aquatic. Bot.* 35, 181-195.

Center, T. D., Cofrancesco, A. F., and Balciunas, J. K. (1990). "Biological control of aquatic and wetland weeds in the southeastern United States." *Proceedings of the VII international symposium on biological control of weeds*. 6-11 March 1988. E. S. Delfosse, ed., Rome, Italy, 239-262.

Center, T. D., Dray, F. A., Jubinsky, G. P. , and Grodowitz, M. J. (1999). "Biological control of water hyacinth under conditions of maintenance management: Can herbicides and insects be integrated?" *Environmental Management* 23, 241-256.

Center, T. D., Grodowitz, M. J., Cofrancesco, A. F., Jubinsky, G., Snoddy, E., and Freedman, J. E. (1997). "Establishment of *Hydrellia pakistanae* (Diptera: Ephydridae) for the biological control of the submersed aquatic plant *Hydrilla verticillata* (Hydrocharitaceae) in the southeastern United States," *Biological Control* 8, 65-73.

Chilton, E. W., III. (1998). "Rio Grande exotic plant status report & action plan," Special Report to the Governor of Texas, Texas Parks and Wildlife Dept., Austin, TX.

Grodowitz , M. J., Center, T. D., Cofrancesco, A. F., and Freedman, J. E. (1997). "Release and establishment of *Hydrellia balciunasi* (Diptera: Ephydridae) for the biological control of the submersed aquatic plant *Hydrilla verticillata* (Hydrocharitaceae) in the United States," *Biological Control* 8, 15-23.

Grodowitz, M. J., Freedman, J. E., Cofrancesco, Al F., and Center, T. D. (1999). "Status of *Hydrellia* spp. (Diptera: Ephydridae) release sites in Texas as of December 1998," Miscellaneous Paper A-99-1, U.S. Army Engineer Research and Development Center, Vicksburg, MS.

Perkins, B. D. (1973). "Release in the United States of *Neochetina eichhorniae* Warner, an enemy of waterhyacinth." *Proceedings. Ann. Meet. S. Weed Science. Soc.* 26, 368.

PMIS. (1998). "Noxious and nuisance plant management information system (CD-ROM)," U.S. Army Engineer Research and Development Center, Vicksburg, MS.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*

2. REPORT DATE
September 2000

3. REPORT TYPE AND DATES COVERED
Final report

4. TITLE AND SUBTITLE
Status of Waterhyacinth/Hydrilla Infestations and Associated Biological Control Agents in Lower Rio Grande Valley Cooperating Irrigation Districts

5. FUNDING NUMBERS
Work Unit No. 33028

6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER
ERDC/EL SR-00-11

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Headquarters, U.S. Army Corps of Engineers, Washington, DC 20314-1000;
U.S. Department of Interior, Bureau of Reclamation, Denver, CO 80225

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*
During September 1999, surveys to quantify plant infestations and insect biological control agents of both *Eichhornia crassipes* and *Hydrilla verticillata* were initiated on the Rio Grande from just west of McAllen, TX, and east to Brownsville, TX. Waterhyacinth biomass/m^2 differed significantly from site to site and ranged from 3 kg/m^2 to about 21 kg/m^2. Both *Neochetina eichhorniae* and *N. bruchi* were commonly collected from all waterhyacinth sites. However, agent numbers were highly variable with an almost fourfold difference in numbers of agents/m^2 observed at the various sites. Strong relationships between various plant parameters and number of insect agents were observed that serve as an illustration of possible insect herbivory effects. For example, at sites with only 20 total *Neochetina* spp./m^2, the number of flower stalks/m^2 averaged about 14 per m^2. This is contrasted to sites with 80 *Neochetina* spp./m^2 where the number of flower stalks/m^2 was reduced over twofold. Hydrilla populations appeared to differ significantly from site to site. While no quantitative measurements of the hydrilla populations were made, visual inspections of the sites revealed hydrilla populations ranging from small, scattered populations to extensive infestations across major portions of the river. In most cases, the hydrilla appeared healthy with no obvious signs of stress. The only insect agent collected from the survey sites was *H. pakistanae*. It was surprising to collect any *H. pakistanae* since the closest release site is over 400 km to the north and populations at this site (Choke Canyon Reservoir) have remained low to nonexistent since the early to mid 1990's. Population numbers on the Rio Grande appeared low with only 300 immatures/kg and about 4 percent of the leaves damaged.

14. SUBJECT TERMS

Biological control	*Neochetina*
Hydrellia pakistanae	Rio Grande
Hydrilla	Waterhyacinth

15. NUMBER OF PAGES
41

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT
UNCLASSIFIED

18. SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

19. SECURITY CLASSIFICATION OF ABSTRACT

20. LIMITATION OF ABSTRACT